

A STUDY ON DEVELOPMENT OF BACTERIAL CONSORTIUM FROM MANGROVE SEDIMENT TO DEGRADE DIBENZOFURAN IN SOIL

YANISWORO WIJAYARATHI

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ABSTRACT

Dibenzofuran is one among polycyclic aromatic hydrocarbons (PAHs) which contains oxygen. As a hydrophobic compound, dibenzofuran tends to be adsorbed by the soil solids, and therefore it is persistent. The discharge of dibenzofuran in the environment needs to be controlled promptly because it is the precursor of its chlorinated derivatives, such as 2,3,7,8 tetrachlorodibenzofuran. Chlorinated dibenzofuran is more toxic than the dibenzofuran itself. Dibenzofuran can be degraded by a single culture of bacteria. However, inhibition occurs in certain steps of the metabolism resulting in a relatively low rate of dibenzofuran degradation.

The present study was conducted to obtain a natural bacterial consortium capable of degrading dibenzofuran rapidly, to formulate an artificial bacterial consortium with dibenzofuran degrading capability equal to the natural consortium, to describe the interaction which take place among members of the artificial bacterial consortium throughout the dibenzofuran degradation process, and to determine factors which play a role in the degradation of dibenzofuran by the bacterial consortium in the soil.

The bacterial consortia were developed from mangrove sediment taken from Balongan, Indramayu, West Java, using liquid minerals medium enriched with dibenzofuran as its sole source of carbon and energy. Soil samples consisted of Vertisol taken from Sragen, Central Java, Latosol taken from Gunung Kidul, Yogyakarta Special Region, and Andosol from Tawang Mangu, Central Java. The remaining dibenzofuran in the medium was analyzed using Gas Chromatography (GC) and the metabolites produced from dibenzofuran degradation were analyzed using GC-MS. Physiological character of component isolates of the artificial consortium were analyzed by BIOLOG EcoPlateTMI or API 20NE bioMerieux, and their identification was based on the 16S rDNA gene sequences.

Four natural dibenzofuran degrading bacterial consortia were obtained from the mangrove sediments. Among the four natural consortia, the A1 consortium had the highest degradation ability. The dibenzofuran degradation rate of the A1 consortium was not significantly different from that of the *Sphingomonas wittichii* RW1, the well characterized dibenzofuran degrading bacteria. A total of 12 bacterial isolates had been isolated from both the A1 consortium and the sediment source of the A1 consortium. Five bacterial isolates, GMYk-1, GMYk-2, GMYk-3, GMYk-4 and GMYk-5 were isolated from the A1 consortium. From the source of the A1 consortium, seven isolates, GMYs-1, GMYs-2, GMYs-3, GMYs-4, GMYs-5, GMYs-6 and GMYs-7 have been obtained. Based on the examination on the similarities of the isolates obtained, and the interaction among the isolates in degrading dibenzofuran, four isolates have been selected. They were GMYs-1,

GMYS-6, GMYS-7 and GMYk-1. Based on the ability of all possible combination of the selected isolates to degrade dibenzofuran, a mixed culture of GMYS-1, GMYS-6, and GMYk-1 was chosen as an artificial consortium. According to the sequence of their 16S-rDNA, the GMYS-1 and GMYS-6 were identified as *Paenibacillus* with 93 and 91% similarities, respectively. The GMYk-1 was identified as *Sphingobacterium* with 97% similarities. The three bacteria were then designated as *Paenibacillus* GMYS-1, *Paenibacillus* GMYS-6 and *Sphingobacterium* GMYk-1.

The artificial consortium had the highest ability to degrade dibenzofuran. The degradation rate of dibenzofuran by the artificial consortium was $167 \text{ mg L}^{-1} \text{ day}^{-1}$, while the degradation rate by the A1 consortium, *Paenibacillus* GMYS-1 and *Paenibacillus* GMYS-6 were 145, 104 and $90 \text{ mg L}^{-1} \text{ day}^{-1}$, respectively. The dibenzofuran degradation rate by *Sphingobacterium* GMYk-1 was very low, i.e. $4 \text{ mg L}^{-1} \text{ day}^{-1}$. During the degradation of dibenzofuran by *Paenibacillus* GMYS-1 or *Paenibacillus* GMYS-6, an accumulation of salicylic acid occurred. The growths of *Paenibacillus* GMYS-1 and *Paenibacillus* GMYS-6 were inhibited by salicylic acid. In contrast, *Sphingobacterium* GMYk-1, which could not metabolize dibenzofuran, metabolized the accumulated salicylic acid. These results show that there was a synergistic metabolite interaction occurring during the dibenzofuran degradation by the artificial consortium.

The artificial consortium was able to degrade dibenzofuran in the Vertisol, Latosol, or Andosol. In Vertisol, the highest dibenzofuran degradation rate by the artificial consortium was $766 \text{ mg L}^{-1} \text{ week}^{-1}$. The highest dibenzofuran degradation rates in Latosol and Andosol were 253 and $141 \text{ mg L}^{-1} \text{ week}^{-1}$, respectively. Partial correlation test between degradation rate with factors that may affect it showed that the dibenzofuran degradation rate in soil was only related to the cell growth rate. Results of the present study demonstrated that cell activity was not affected by soil organic matter content.

Key words: Degradation, dibenzofuran, bacterial consortium, metabolites, soil.